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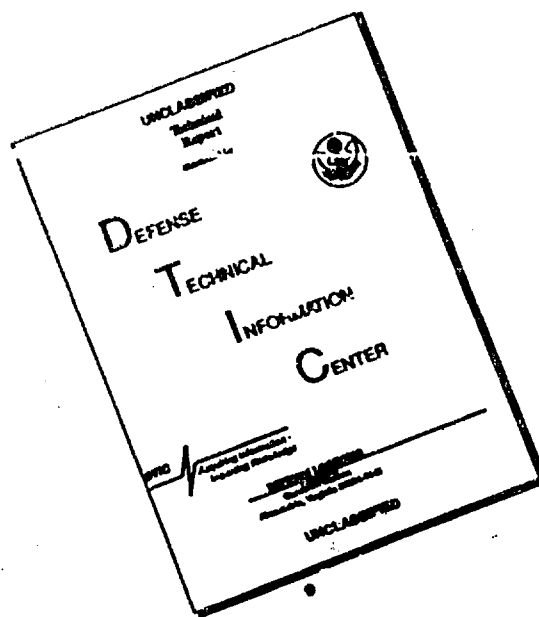
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Dry Medium for the Diagnosis of Food Poisoning Caused by Clostridium
Botulinum and Clostridium Perfringens

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Magnificent achievements were accomplished in our country in the period following the Great October Socialist Revolution, namely in the improvement of the food production technology and in the advancement of the sanitary operation of food enterprises. These measures contributed to a complete elimination of large-scale outbreaks of food poisonings among the population and, particularly, those of botulinal poisonings.

It has been assumed for a long time that, considering a group of anaerobic microorganisms, only Cl. botulinum is active in the etiology of food poisonings in man. But, in recent years, reports appeared in ever increasing number in the literature about the instances of toxinfections caused by Cl. perfringens (2 to 5).

Extensive investigations carried out in England, France and in other countries proved that Cl. perfringens assumed a third place as a causative agent of food poisonings, succeeding Salmonella and Staphylococci microorganisms.

One of the reasons of unsatisfactory bacteriological diagnoses in cases of alimentary toxoinfections caused by anaerobes is the fact that the standard-culture media, suitable for growing these micro-organisms, are not available in the laboratories at the right places. The TAROZZI medium, used extensively for these purposes in test tubes, is unsuitable for proper growth and for the production of toxin of all types of causative agents of botulism and *Cl. perfringens*. Furthermore, the microbiological practice during the preparation of media is diversified in individual laboratories and, frequently, lacks the observance of essential conditions and this, in turn, leads to the instability of the media; yet, it is impossible to arrange a central supply point of liquid media due to the transportation difficulties and the problems of storage. Thus, in order to meet the demands of diagnostic laboratories, it is necessary to have dry media available that would be conveniently transported and produced according to a consolidated procedure.

The objective of our research was to produce, from technological products, a dry serum, which would be simple in preparation and useful for diagnosis of food poisonings that are caused by *Cl. botulinum* types A, B, C and E, also by *Cl. perfringens* types A, B, C, D, E and F. We prepared and tested 211 specimens of the media, analysing them by their composition and handling techniques. We made a preliminary rating of each specimen following the inoculation of *Cl. perfringens* type A, strain No.28, on 10 ml of medium under vaseline oil, with a subsequent arrangement of the lecithovitellin test for the presence of lecithinases. In the event of a positive

reaction, the specimens in question were tested by way of inoculation of *Cl. perfringens* types A, B, C, D, E and F, also of *Cl. botulinum* types A, B, C and E, with a subsequent titration of the toxin's strength on white mice.

The titration of toxins of *Cl. botulinum* type E and of *Cl. perfringens* types D and E was carried out after a preliminary activation of the culture fluid by means of an equal volume of a 4% solution of medical pancreatin, in accordance with the method described by K.I. MATVEEV and B.D. BYCHENKO.

We used for control purposes a casein-fungal medium No.3 (I. N. VINOGRADOVA, originator). The strength of the toxins produced with this medium was evaluated by tracing them on white mice with the aid of the neutralization reaction.

As a basic culture medium we used a cetacean meal, which, as such, is a technological product with a high protein content. The fermentative hydrolysates prepared from the cetacean meal can be filtered well and fast; they yield transparent filtrates that contain at least 15 different amino acids. Yet, we failed to produce sufficiently strong toxins of all types of microorganisms while using one tryptic digest of the cetacean meal to grow *Cl. perfringens* and *Cl. botulinum*.

According to reported data, a fungal extract (yeast water) may act on bacteria as a source of the germination factors. We increased the toxic yield of *Cl. botulinum* type C to 10,000 Dlm/ml by adding yeast water to the tryptic hydrolysate of cetacean meal. Then, when we added to the medium either liver water, or minced

Table 1

Production of Toxins of Cl. Botulinum and Cl. Perfringens in the
Cetacean-Meal (KPD) Medium and in the Control Medium

Culture	KPD (liquid)	KPD (dry)	Casein-fungal control medium
	Dlm/ml		
<u>Cl. perfringens:</u>			
A-28	50	50	50-100
B-216	500	500	200-500
C-219	2000	2000	500-1000
D-2	1000	1000	500-1000
E-342	10	10	10
P-48	200	200	100-500
<u>Cl. botulinum:</u>			
A-Memphis	100,000	100,000	100,000
B-175	100,000	100,000	100,000
C-91	10,000	10,000	10,000
E-188-20	1,000	1,000	100-1000

liver without yeast water, we failed to produce the same effect. The best results were obtained after addition of yeast water to the fermentative hydrolysate of minced liver (cetacean liver medium with yeast fungi — KPD).

While finishing off the KPD medium we discovered that it is more rational to add yeast as a suspension in a ratio of 1:2 prior to the filtration of alkaline hydrolysate (pH 7.8 to 8.0). The duration of hydrolysis was 40 minutes. We added, to the medium, salts of phosphate sodium and potassium, also a small quantity of

magnesium salt and, prior to using it, we added 0.5% of glucose.

In order to inoculate the medium, we poured out 70 ml volume into each flask of 100 ml capacity with a wadding and we sterilized this for 30 minutes at 110°C. An increase in the sterilization temperature reduced the quality of the medium. We added to the medium 3.5 ml of vaseline oil per each flask.

We quote in Table 1 some data indicating the strength of toxins in KPD media after inoculation of *Cl. botulinum* types A, B, C and E, also of *Cl. perfringens* types A, B, C, D, E and F, as compared with the control medium of casein fungi.

Thus, on the basis of obtained data, we were^{able} to use this medium as a sample in order to obtain the same substance in a dried form.

The formula for preparation of the KPD medium was as follows: cetacean meal 150 gm; minced liver 100 gm and pancreatin 10 gm per 1 l of tap water heated to 50°C.

The hydrolysis was carried out at 50° temperature. The starting time of hydrolysis was recorded at once following the determination of the hydrolytic mixture pH at 7.8. The alkalization was effected with a 40% solution of caustic soda. In 40 minutes after the beginning of hydrolysis, as the content of nitric amine attained by hydrolysis 240 mg% (no more!), the hydrolysis was discontinued with the addition of concentrated hydrochloric acid up to the pH = 4.0, then the mixture was brought to a boiling point and was subsequently filtered. Having 800 ml of produced filtrate, the latter was alkalized again with caustic soda up to the pH=8.0, then we added 3.4 gm of disubstitution products of sodium phosphate, 0.45

gm of monosubstitution products of potassium phosphate, 0.03 gm of magnesium sulfate and 70 gm of yeast diluted in 140 ml of distilled water. This was boiled for 5 minutes and then filtered until the formation of transparent fluid of light brown color. Thus produced KPD medium, before being used, was diluted to the content of 150 to 170 mg% of nitric amine and then tested bacteriologically. The indicated content of nitric amine in the KPD medium contained a total of 550 to 570 mg% of nitrogen, 55 to 60 mg% of albuminous nitrogen, 5 to 7% of peptone, 75 to 80 mg% of total phosphorus, 55 to 57 mg% of inorganic phosphorus, 1.0 to 1.2% of tryptophan, 0.7 to 0.8% of chlorides and 1.0 to 1.2% of ash content.

This medium is suitable for the growth and production of toxins of *Cl. perfringens* types A, B, C, D, E and F, also of *Cl. botulinum* types A, B, C and E. It preserves its qualities after evaporation and desiccation.

In order to produce 1 l of ready KPD medium, it is required: 165 gm of oetacean meal; 110 gm of minced liver; 11 gm of pancreatin; 20 ml of a 40% solution of caustic soda; about 17 ml of concentrated hydrochloric acid; 77 gm of bread yeast; 0.5 gm of monosubstitution products of potassium phosphate; 3.75 gm of disubstitution products of sodium phosphate; 0.033 gm of magnesium sulfate and tracers of universal and bromothymol blue dyestuffs.

We obtained a medium of industrial specifications in a quantity of 100 l, delivered in a reaction vessel of 150 l capacity. We concentrated the medium by evaporation up to 55 or 60% of dry

residue and then we desiccated the latter in a vacuumtight roller drier. We obtained about 5 gm of dried medium. The latter resembled a powder of bright yellow color, with a moisture content of 3.5 to 5%, and it was packed in a safely secured vessel, hermetically closed. The medium was stored at room temperature and thus it did not deteriorate in quality for one year.

We used for preparation of the medium (broth) 7 gm of dry powder per 100 ml of distilled water and, while stirring, we brought this to a boiling point. If needed, we adjusted the reaction up to the pH 7.8 or 8.0 by adding 0.25 to 0.5% of glucose. The prepared broth was poured out in a 70 ml volume into each flask of 100 ml capacity with a wadding, then we added 3.5 ml of vaseline oil to each flask and we sterilized this for 30 minutes at 110°C.

Using this medium and following the inoculation of *Cl. botulinum* types A, B, C and E, also of *Cl. perfringens* types A, B, C, D, E and F, we determined the strength of toxins in accordance with the aforesaid method for a liquid medium. As to its qualities and bacteriological index, the dried KPD medium showed no difference from the liquid medium (see Table 1).

We also tested the dry medium in connection with the isolation of *Cl. botulinum* from the organs of dead animals infected with causative agents of botulism. We infected guinea pigs by administering into their stomach 1, 2 and 5 Dlm doses of *Cl. botulinum* types A, B, C and E. Following the death of animals, we inoculated their liver, also large and ^{small} intestines on a broth prepared from the dry medium and on the casein-fungal medium for control purposes.

After 48 hours, we determined the strength of botulinal toxins in both media by titration on white mice with the antibotulinal serum of the corresponding type.

The results of the inoculation on the dry medium and on the control medium were the same; this indicates that the dry medium can be used for inoculation to determine the presence of Cl. botulinum in the organs of cadavers.

Conclusions

1. The produced KPD dry medium is suitable for diagnosis of food poisonings caused by Cl. botulinum types A, B, C and E, also by Cl. perfringens types A, B, C, D, E and F.

The growth and the production of toxins of the indicated causative agents proved to be the same as on the nutrient media that are used for growing these bacteria.

2. The simplicity in the preparation of the KPD medium from a dry specimen permits to use the latter in any laboratory. The medium can be conveniently stored and transported.

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